Standard Extraction Method
PRIOR ART

Dry lipids under nitrogen

⇓

Sonicate in buffer to form micelles
Add reaction mixture

⇓

Stop Reaction

⇓

Extract lipids in chloroform/methanol
to induce two phases

⇓

Spin down

⇓

Remove upper (water) phase

⇓

To lower phase add
artificial upper phase

⇓

Spin down

⇓

Remove lower (lipid) phase
into fresh tubes

⇓

Count or run TLC

Present Invention

Spot liquids on the membrane directly
from chloroform/methanol solution

⇓

Add reaction mixture
(enzyme + ^{32}P-ATP)

⇓

Stop reaction

⇓

Wash membranes

⇓

Phosphoimage analysis or
radioactivity counting

FIG. 1

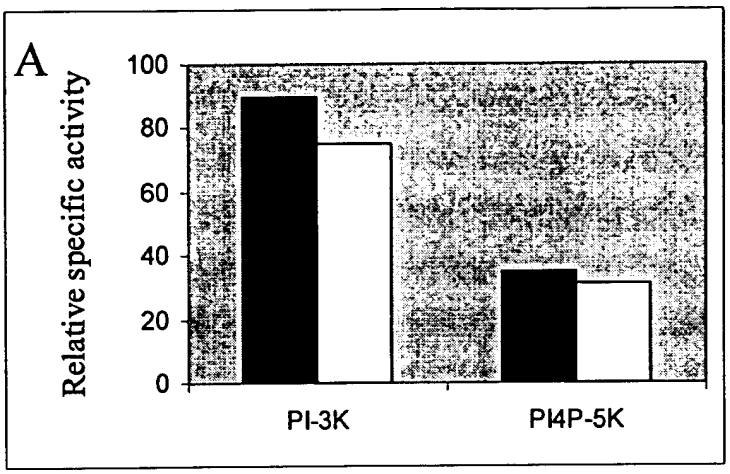
A
Relative specific activity
100
80
60
40
20
0
PI-3K
PI4P-5K

FIG. 2A

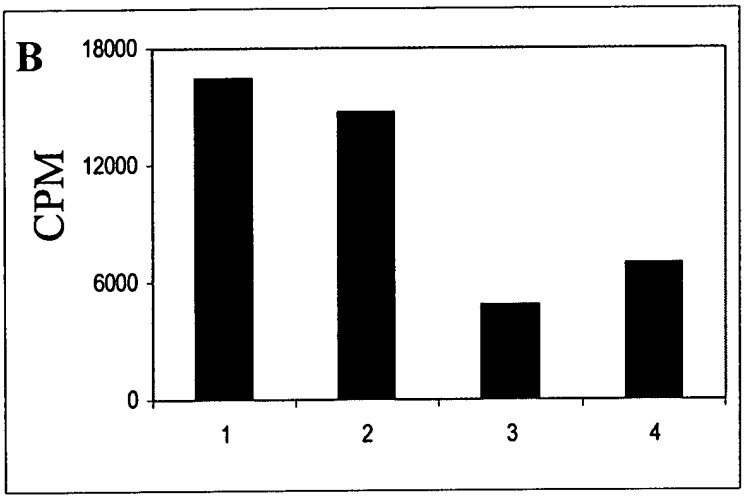
B
CPM
18000
12000
6000
0
1
2
3
4

FIG. 2B

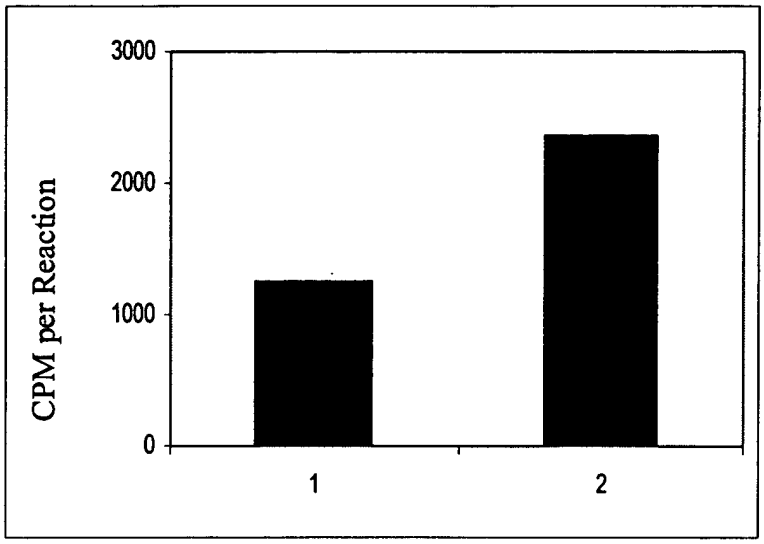

FIG. 3

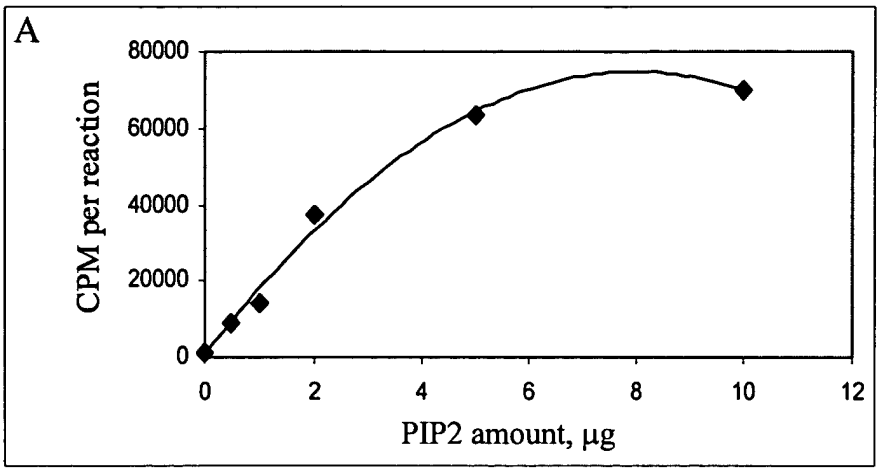
A
80000
60000
40000
20000
0
CPM per reaction
0
2
4
6
8
10
12
PIP2 amount, µg

FIG. 4A

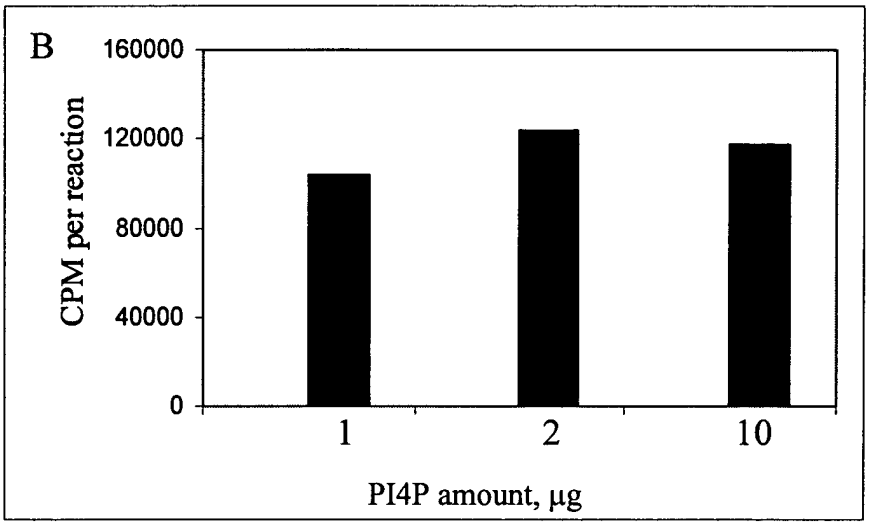
B
160000
120000
80000
40000
0
CPM per reaction
1
2
10
PI4P amount, µg

FIG. 4B

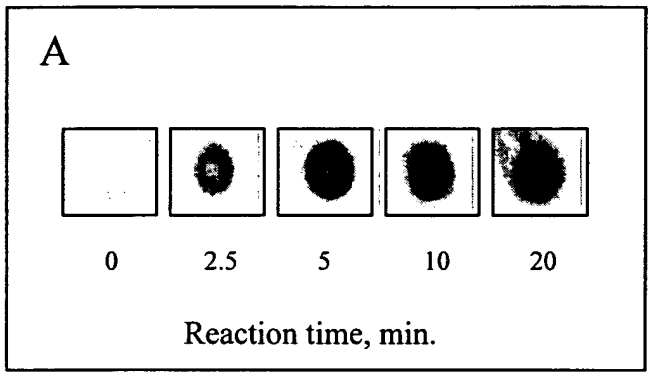

FIG. 5A

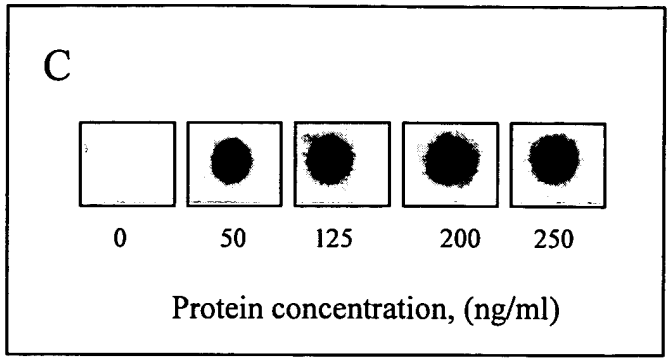

FIG. 5C

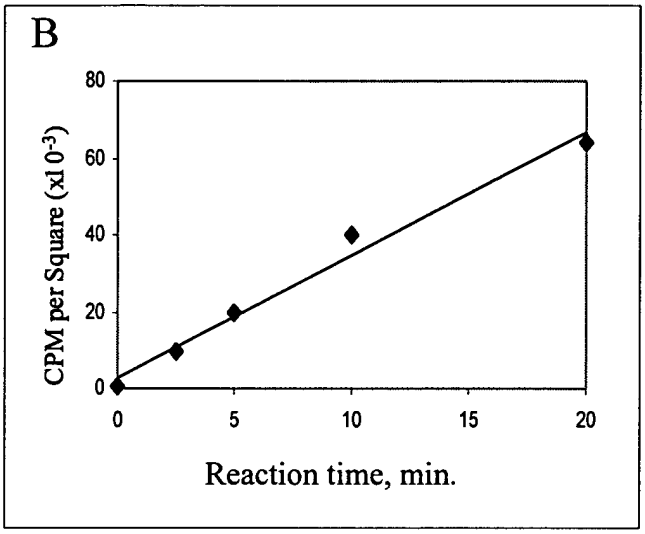

FIG. 5B

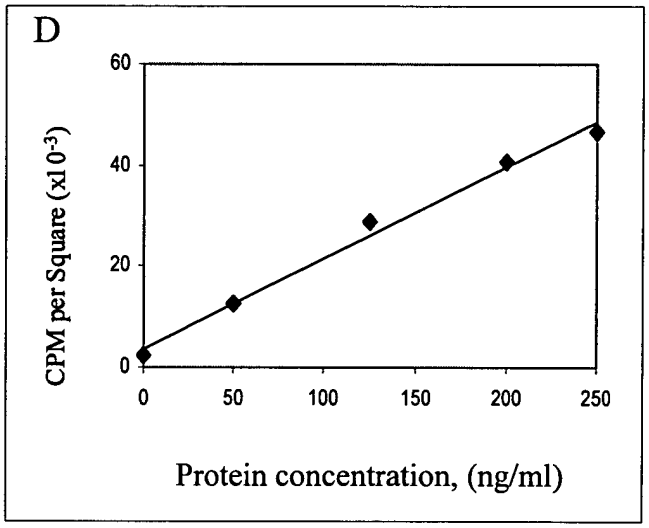

FIG. 5D

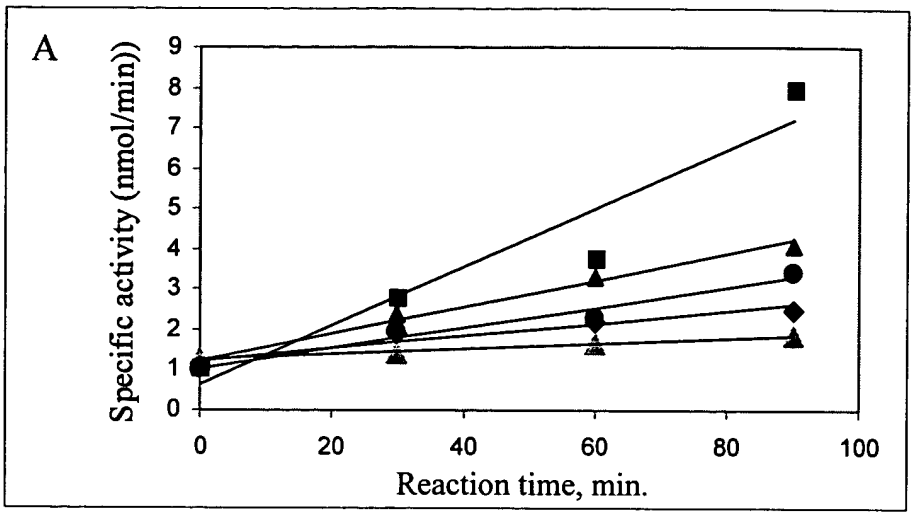
A
Specific activity (nmol/min))
0
1
2
3
4
5
6
7
8
9
0
20
40
60
80
100
Reaction time, min.

FIG. 6A

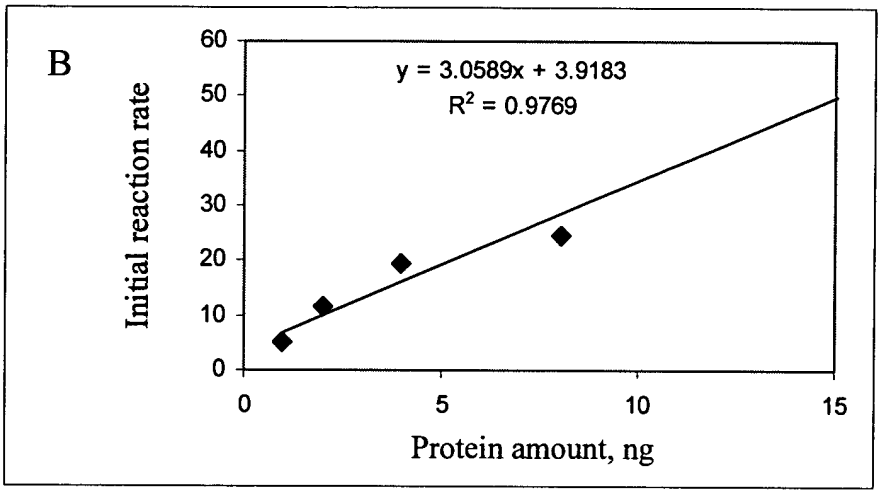
B
Initial reaction rate
y = 3.0589x + 3.9183
R2 = 0.9769
0
10
20
30
40
50
60
0
5
10
15
Protein amount, ng

FIG. 6B

Main points: 1. Reproducibility

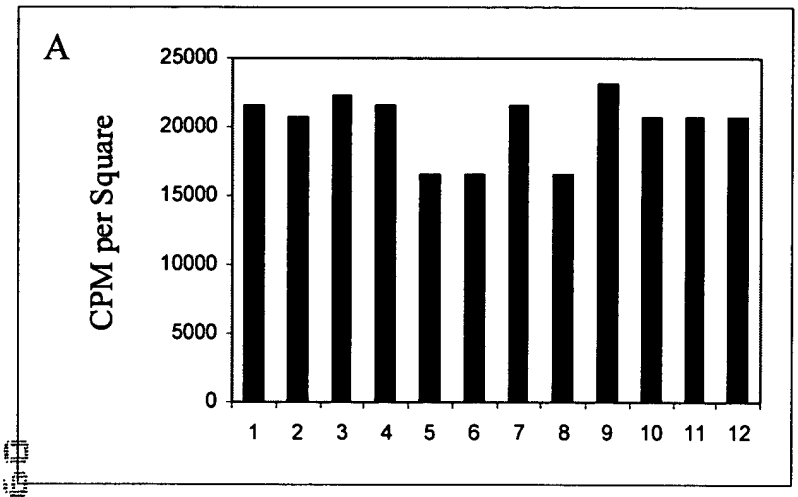

FIG. 7A

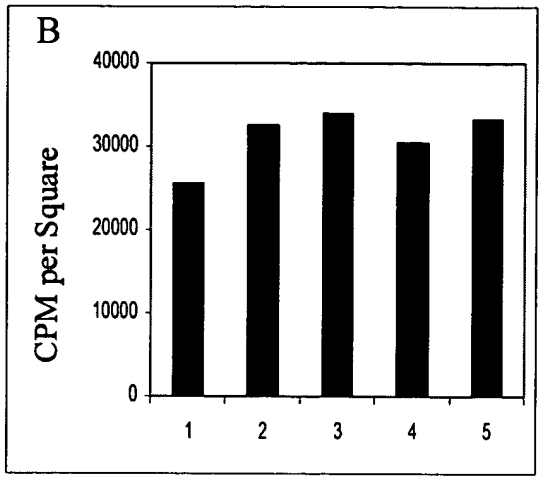

FIG. 7B

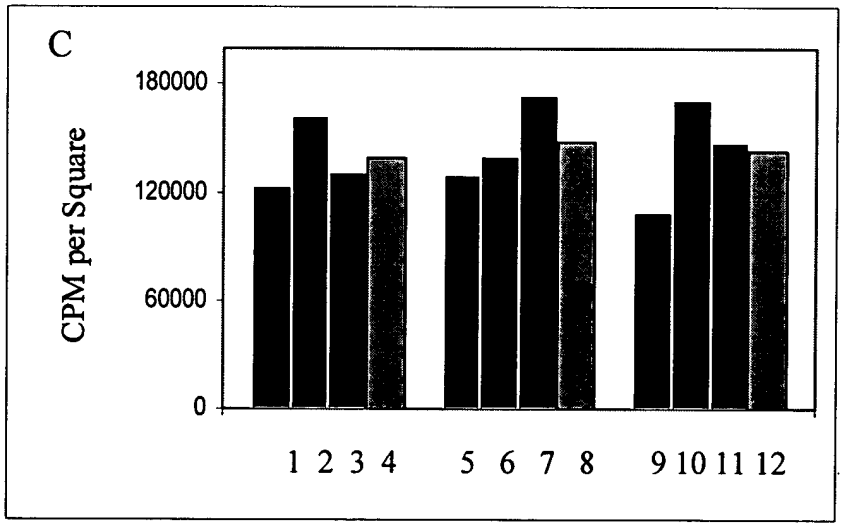

FIG. 7C

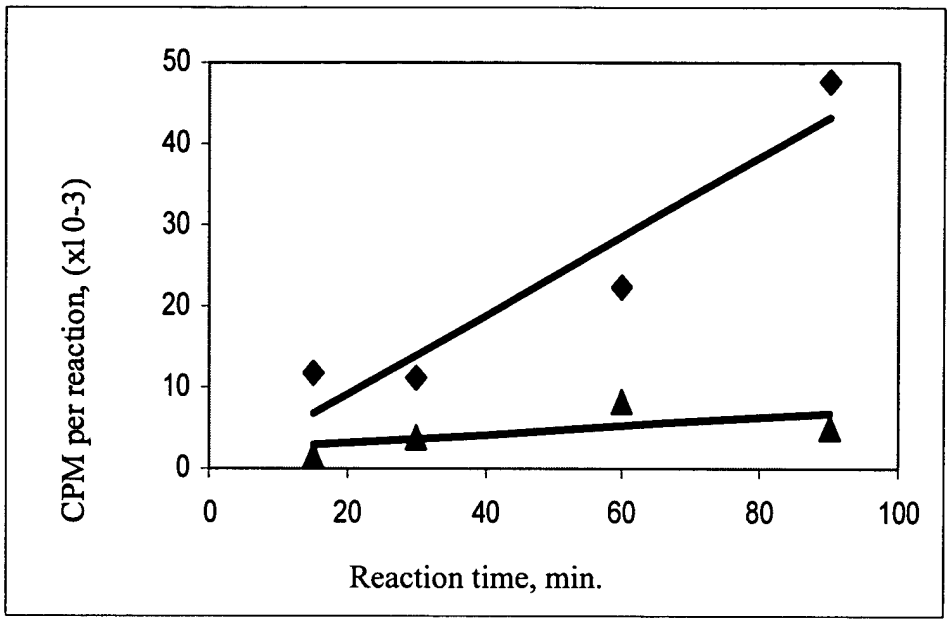
50
40
30
20
10
0
CPM per reaction, (x10-3)
0
20
40
60
80
100
Reaction time, min.

FIG. 8

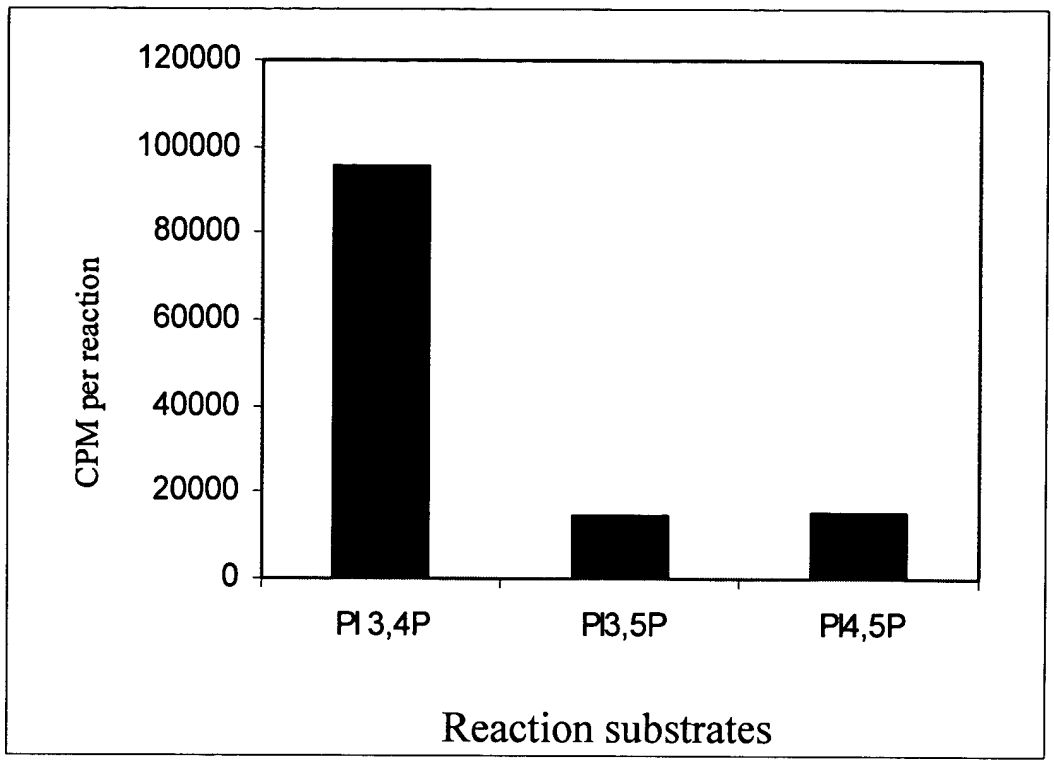
120000
100000
80000
60000
40000
20000
0
CPM per reaction
PI 3,4P
PI3,5P
PI4,5P
Reaction substrates

FIG. 9

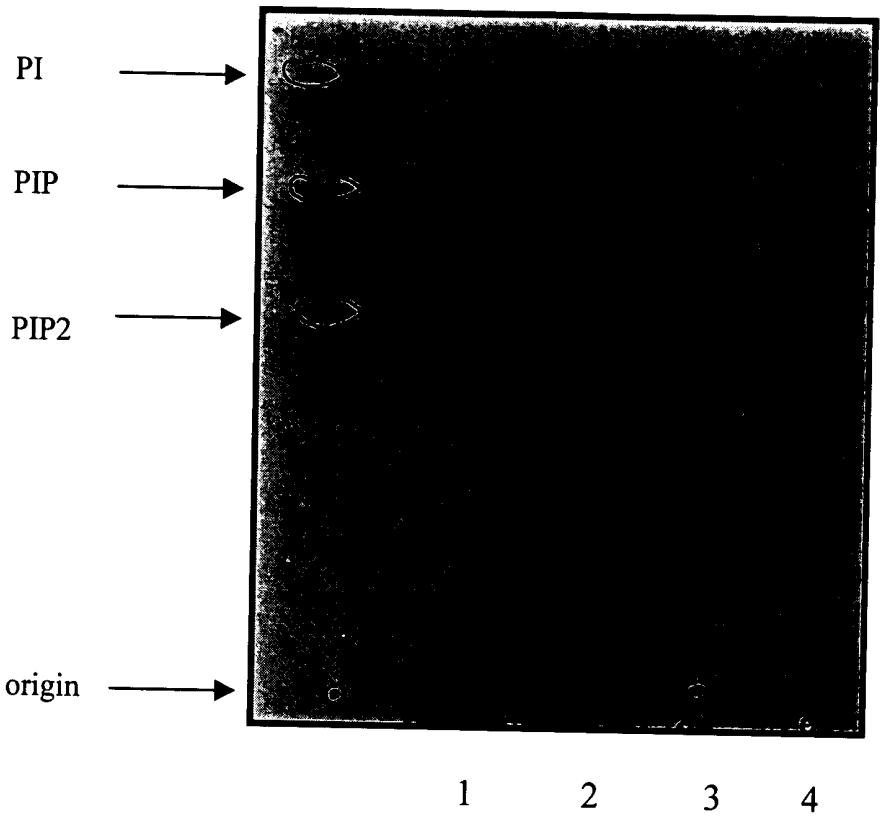

FIG. 10

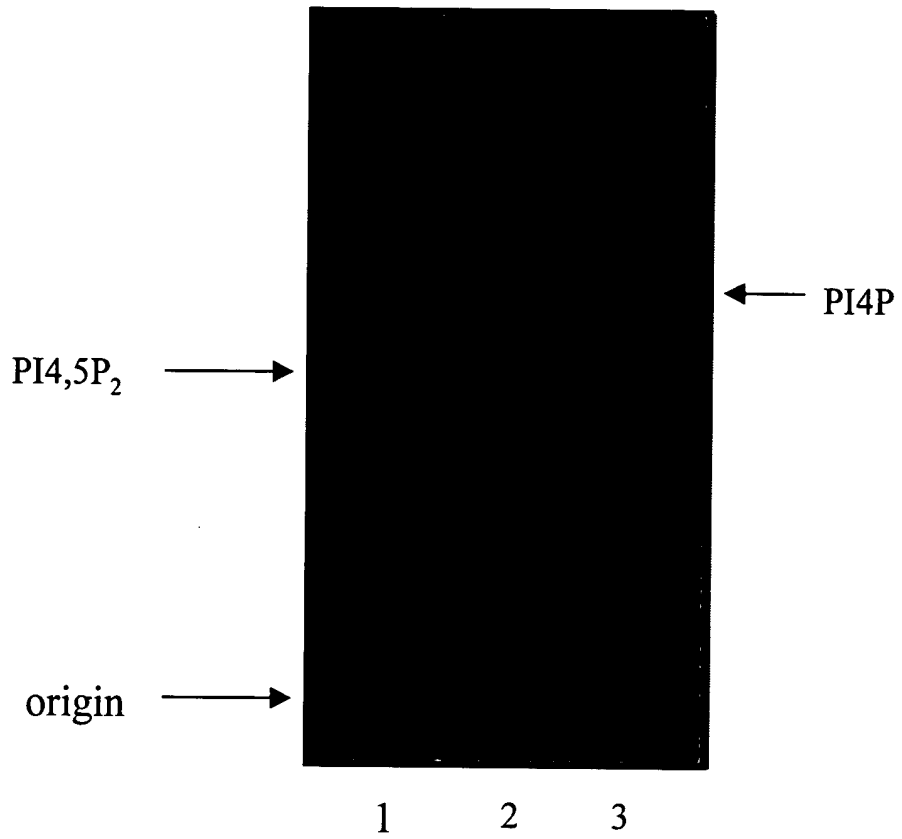

FIG. 11A

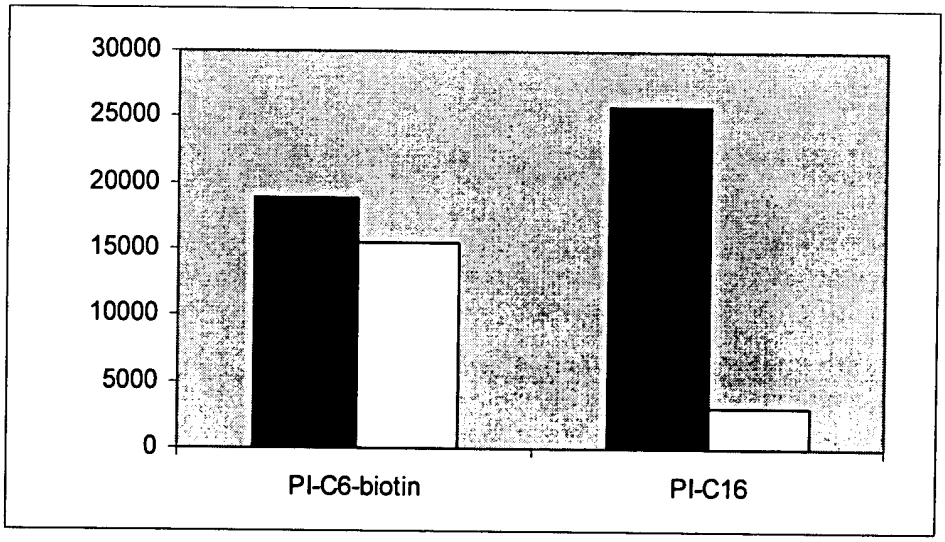

FIG. 11B